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Myocardial ischaemia/reperfusion is associated with mitochondrial dysfunction followed by the cardiomyocyte death. If ischaemia is followed by rapid reperfusion it may cause cellular dysfunction and tissue injury. Mitochondria are recognized as key organelle involved in post-ischemic reperfusion damage.

Mitochondrial complex I can exist in two interconvertible states: active (A) and de-active or dormant (D). We have studied the A/D equilibrium in several tissues after in vivo induced ischaemia and investigated the sensitivity of both forms of the enzyme to reactive oxygen species (ROS).

We performed analysis of the A/D ratio of mitochondrial complex I obtained from various tissues after the onset of cardiac arrest. We found that in highly metabolising tissues such as heart and brain the  $t_{1/2}$  of complex I de-activation is 12 and 2.5 min, respectively. Reperfusion resulted in the return of complex I A/D equilibrium to its initial level, indicating that reintroduction of oxygen causes reactivation of the D-form of the enzyme. The generation of excessive quantities of ROS and subsequent tissue damage is an important mechanism of reperfusion injury. We found that only the D-form of complex I was susceptible to inhibition by ROS whereas the A-form of the enzyme was insensitive to either hydrogen peroxide or superoxide. Turnover-dependent activation of the preparation D-form of the enzyme resulted in formation of the A-form which was much less sensitive to ROS. The nature of complex I modification by ROS is likely to involve oxidation of cysteine thiols. The D-form of complex I modified in this way does not catalyse the physiological NADH:ubiquinone reaction, making this enzyme a mitochondrial target for oxidative stress and an early target in ischaemia/reperfusion damage. Therefore, transition of complex I into the dormant form in the absence of oxygen is critical to the process of cardiac injury during ischaemia/reperfusion and may have important pathophysiological consequences.

doi:[10.1016/j.bbabbio.2012.06.310](https://doi.org/10.1016/j.bbabbio.2012.06.310)

## 15L4

### ROS production by respiratory complex II

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A common observation is that the rate of  $H_2O_2$  production is remarkably higher under succinate oxidation than under NADH-linked (glutamate/pyruvate) oxidation in isolated rat brain, heart and skeletal muscle mitochondria. As the  $H_2O_2$  production driven by succinate oxidation is strongly inhibited by the CI inhibitor rotenone, it is generally believed that succinate oxidation produces ROS via a "reverse electron flow" from CII to CI. However, oxidation of submillimolar (physiological) concentrations of succinate can produce  $H_2O_2$  even in the presence of high concentrations of NAD-dependent substrates or NADH, which makes the presumed CII-CI reverse electron flow more thermodynamically unfavorable.

Moreover, although rotenone potentially block ROS production driven by succinate oxidation, approximately 10–15% of the initial ROS remains, which cannot be catalyzed by CI FMN $H_2$ . In addition, this ROS fraction may be significantly higher (1.3–15 times) than the

ROS produced by the CI forward reaction. The simpler explanation for the generation of the rotenone-insensitive ROS driven by succinate oxidation is that they are produced by the CII redox components. However, it is surprising to find that CII contribution to ROS production has been mostly disregarded and hence has not been experimentally assessed. In the present study, experiments were designed to undoubtedly establish that CII indeed significantly contributes to the generation of ROS.

doi:[10.1016/j.bbabbio.2012.06.311](https://doi.org/10.1016/j.bbabbio.2012.06.311)

## 15L5

### Functional characterization and regulation of UCP4 expression by adipokinetic hormone in larva and pupa fat body mitochondria from the beetle *Zophobas atratus*

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Uncoupling protein 4 (UCP4) is the member of the large family of mitochondrial anion transporters that uncouple oxidative phosphorylation [1]. The present study demonstrates for the first time, the molecular identification of a partial *Zophobas atratus* UCP4 coding sequence and the functional characterisation of ZaUCP4 in the mitochondria of larval and pupal fat bodies of the beetle. ZaUCP4 shows a high similarity to predicted insect UCP4 isoforms and known mammalian UCP4s, both at the nucleotide and amino acid sequence levels. Bioenergetic studies unequivocally demonstrate UCP4 activity in mitochondria isolated from larvae and pupae fat body. In resting, non-phosphorylating state 4 respiration ZaUCP activity was stimulated by palmitic acid and inhibited by the purine nucleotide GTP. In phosphorylating mitochondria, ZaUCP4 activity decreased the yield of oxidative phosphorylation. ZaUCP4 was immunodetected by using of antibodies raised against human UCP4 as a single 36 kDa band. Because it is known that hormones influence an expression of UCPs, we tested adipokinetic hormone (AKH), analog of mammal glucagon, which mobilizes lipids and carbohydrates from fat body stores [2]. Besides this energy-mobilizing function, AKHs inhibit fat body lipid and protein synthesis. After AKH injection, we observed the decrease in ZaUCP4 expression at the mRNA and protein levels in both developmental stages of the beetle.

Real time analysis, immunological detection and bioenergetic characteristic indicate consistently for the higher expression of UCP4 in the *Z. atratus* larval fat body compared with the pupal fat body, furthermore ZaUCP4 expression is under hormonal control. The different expression patterns and activity of ZaUCP4 during the larval-pupal transformation indicate an important physiological role for UCP4 in insect fat body development and function during insect metamorphosis. Furthermore, the regulation of UCP4 expression by AKH indicates that it may play a role in maintaining metabolic homeostasis in the insect.

This work was supported by the grant of the Polish Ministry of Science (N N 303291634).

## References

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doi:[10.1016/j.bbabbio.2012.06.312](https://doi.org/10.1016/j.bbabbio.2012.06.312)